

*Recommendation from the Scientific Committee on  
Occupational Exposure Limits  
for 2-Methoxyethanol and 2-Methoxyethyl Acetate*

8-hour TWA:	1 ppm
STEL (15 min):	-
BLV:	8 mg MAA per gram creatinine, in urine sampled at the end of work week after at least two weeks at work
Additional classification:	Skin notation

*Substance Identity and Properties*

*2-Methoxyethanol (2ME)*

CAS No.:	109-86-4
Synonyms:	ethylene glycol monomethyl ether (EGME), methyl glycol
Formula:	$\text{CH}_3\text{-O-CH}_2\text{-CH}_2\text{-OH}$
Molecular weight:	76.09
Density:	0.96 (20°C)
Boiling point:	124°C
Melting point:	-85.1°C
Vapour pressure:	1.3 kPa (9.7 mm Hg) (20°C)
Evaporation rate:	0.5 (butyl acetate = 1)
Saturation concentration:	12,800 ppm (25°C)
Relative density:	2.6 (air = 1)
Conversion factors:	1 ppm = 3.11 mg/m <sup>3</sup> (20°C)

1 mg/m<sup>3</sup> = 0.322 ppm (20°C)

EU-Classification: R10 (Flammable)  
Repr. Cat. 2; R60-61 (May impair fertility and may cause harm to the unborn child)  
Xn; R20/21/22 (Harmful by inhalation/in contact with skin/if swallowed)

*2-Methoxyethyl acetate (2MEA)*

CAS No.: 110-49-6  
Synonyms: ethylene glycol monomethyl ether acetate (EGMEA), methyl glycol acetate  
Formula: CH<sub>3</sub>-O-CH<sub>2</sub>-CH<sub>2</sub>-O-CO-CH<sub>3</sub>  
Molecular weight: 118.13  
Density: 1.005 (20°C)  
Boiling point: 145°C  
Melting point: - 65°C  
Flash point: 55.6°C (open cup)  
Vapour pressure: 0.27 – 0.50 kPa (2.0 – 3.7 mm Hg) (20°C)  
Evaporation rate: 0.3 (butyl acetate = 1)  
Saturation concentration: 3100 – 6000 ppm (25°C)  
Relative density: 4.07 (air = 1)  
Conversion factors: 1 ppm = 4.90 mg/m<sup>3</sup> (20°C)  
1 mg/m<sup>3</sup> = 0.200 ppm (20°C)  
EU-Classification: R20/21/22 Harmful by inhalation/in contact with skin/if swallowed  
R60 May impair fertility  
R61 May cause harm to the unborn child

This summary document is based on a criteria document (Johanson, 1999, 2000), a subsequent consensus document from the Swedish Criteria Group for Occupational Standards (Montelius, 2000), updated with more recent publications covering the period 1999-mid 2004.

2ME and 2MEA at room temperature are flammable, volatile, clear liquids with a weak, sweetish odour and bitter taste. Both substances dissolve readily and completely in water as well as polar and non-polar solvents.

2ME is produced by a reaction between methanol and ethylene oxide. 2MEA is produced from 2ME by conventional esterification. Known impurities in 2ME are reported to be < 0.1% methanol, <0.1% diethylene glycol methyl ether and <0.02% ethylene glycol.

### Occurrence and Use

2ME and 2MEA do not occur in nature. World-wide reported uses for the two glycol ethers are in paints and enamels; printer's ink; plastic packaging for foodstuffs; pigments for silk-screen printing; photographic and photolithographic processes (including the production of offset plates); CDs, circuit boards and integrated circuits; cleaners for household and industrial use; and antifreeze in hydraulic fluids and airplane fuel. In 1994 2ME and 2MEA were classified by the EU as toxic to reproduction and their use in consumer products was prohibited. The usage has declined significantly. For example, according to the Swedish Chemical Products Register, the annual use of 2ME dropped from 260 tons in 1993 to 19 tons in 1997. The annual use of 2MEA in Sweden was reportedly less than 0.1 t/y in 1997.

Reported average exposure levels are in the range <0.1 to 23 mg/m<sup>3</sup> (<0.3 to 7.4 ppm) for 2ME, and from <0.1 to 143 mg/m<sup>3</sup> (<0.2 to 29 ppm) for 2MEA. Exposure has been reported from semiconductor and circuit board manufacture, printing, painting (especially automobile and ship painting), furniture finishing, paint production and automobile repair.

### Health Effects

#### **Uptake, biotransformation and excretion**

As suggested by the chemical structure and the high solubility of 2ME and 2MEA, both substances are efficiently absorbed via all routes and rapidly distributed throughout the body. The respiratory uptake has been measured at 76% of the amount inhaled.

Uptake of 2ME by frozen and thawed human epidermis in vitro was 2.8 mg/cm<sup>2</sup>/h (Dugard et al., 1984).

An average absorption rate of 2.9 mg/cm<sup>2</sup>/h, with large inter-individual variations, was measured for liquid 2ME in an in vivo study with volunteers. Exposure of hands and lower arms to EGME in liquid form was calculated to yield an absorption rate 100 times that of exposure to 5 ppm in the air. The authors also calculated that, with whole-body exposure to EGME vapor, 55% of total uptake occurs via the skin (Kezic et al., 1997).

Applying the ECETOC criteria (exposed area skin area 2000 cm<sup>2</sup>, skin exposure duration 1 h, inhaled volume 10 m<sup>3</sup> in 8 h) on the Kezic et al. data, the daily dermal dose of 5 800 mg would be equivalent to 580 mg/m<sup>3</sup> (186 ppm) EGME.

In another single-arm exposure of seven volunteers to EGME vapours at 25 or 300 ppm, percutaneous absorption rates of 1.4 and 13 µg/cm<sup>2</sup>/h were obtained (Shih et al., 2000b). Extrapolating these data to whole-body exposure to EGME vapours, 17-20% of the total dose would be absorbed via skin (skin area 1.8 m<sup>2</sup>, pulmonary ventilation 10 m<sup>3</sup>/8 h).

2ME is distributed fairly evenly between blood and other tissues, with the exception of low solubility in adipose tissue. Methoxyacetic acid (MAA), a major metabolite of both 2ME and 2MEA, also has relatively even distribution in body tissues.

2MEA is efficiently hydrolyzed to 2ME by the carboxylesterases present in the nasal mucosa, liver, kidneys, lungs and blood. The most important metabolic pathway for 2ME is oxidation via methoxyacetaldehyde (MALD) to MAA. This metabolism can be inhibited by ethanol, and the importance of alcohol dehydrogenase is illustrated by the fact that metabolism of 2ME is almost completely suppressed in rats that have been pre-treated with pyrazol. When men were exposed to 5 ppm 2ME for 4 h (resting), an estimated 86% of the inhaled amount of 2ME was excreted in urine as MAA. The reported half time for MAA in human urine is 77 h. The half time for MAA in serum and plasma has been reported to be about 6 h for mice and 20 h for monkeys.

In addition to MAA, methoxyethyl glucuronide, methoxyethyl sulphate, ethylene glycol, glycolic acid, glycine, methoxyacetyl glucuronide, methoxyacetyl glycine, methoxycitrate and methoxybutenic acid were identified by nucleomagnetic resonance spectrometry (NMR) analysis of urine samples from mice and rats treated with <sup>13</sup>C-labelled 2ME. Simultaneous administration of acetate, an endogenously formed substance and a precursor in the Krebs cycle, increased the proportion of 2ME-related metabolites and reduced the proportion of MAA-related metabolites. These results show that ether cleavage can occur, and also that 2ME after oxidation can form methoxyacetyl-coenzyme A. It has been suggested that this “false substrate” in the Krebs cycle may be related to the toxic effects of 2ME on reproduction .

### **Biological exposure monitoring**

It is difficult to use data from work places to establish relationships between external exposure to 2ME and urinary excretion of MAA since dermal absorption may have contributed significantly to the total dose in these cases. Further, none of the hitherto published physiologically-based toxicokinetic models for 2ME are useful in respect. However, there is one experimental study by Groeseneken et al. (1989) that may be used.

The uptake and disposition of 2ME was studied in seven male volunteers during experimental to 5 ppm 2ME for 4 x 50 min at rest. The administration of 2ME vapour was by mask, thus no dermal exposure occurred. The average urinary elimination half time of MAA was 77.1 h and the total amount excreted was calculated by extrapolation to 86% of the inhaled dose (Groeseneken et al., 1989).

Based on the Groeseneken study a NIOSH report estimated that 8 h exposure to 0.1 ppm 2ME would approximate 0.8 mg MAA/g creatinine at end of shift.

Using a simple pharmacokinetic one-compartment level and one workweek (5d x 8h) of exposure at 1 ppm, the Groeseneken data would correspond to approximately 6-9 mg/g cr at Friday end of shift after the first week, 7-11 mg/g cr after the second week and 8-12 mg/g cr after several weeks of exposure. Assuming several weeks of exposure the predicted increase from Monday morning before shift to Friday end of shift is 3-5 mg/g cr (see appendix).

In a study by Shih et al. (2000a) in Taiwan, 8-h personal breathing zone samples and urine samples before and after shift were collected from Monday to Saturday from 27 workers exposed to 2ME and on Friday from 30 control workers. No urinary MAA was detected in

workers in the non-exposed control group. For 18 regular operation workers not using personal protective equipment, a significant correlation ( $r = 0.702$ ,  $p = 0.001$ ) was found between urinary MAA on Friday after shift and weekly mean exposure to 2ME. The regression equation indicated that 5 d x 8 h exposure at 1 ppm 2ME corresponds to 8 mg MAA/g creatinine. A significant correlation was also found between the weekly increase of urinary MAA (Friday after shift minus Monday before shift) and the weekly mean exposure of 2ME. In this case, 1 ppm corresponded to 4 mg MAA/g creatinine.

Similar results were reported by Chang et al. (2004) in a study primarily aimed to evaluate the effectiveness of gloves during occupational exposure to 2ME. In a group of 25 workers involved in special operations (raw material mixing, charging, machine cleaning), with limited use of gloves, the average 2ME level in air was 8.1 ppm, while MAA at end of Friday shift was on average 73 mg/g Cr. This corresponds to 9 mg/g Cr at 1 ppm. However, a group of 49 less exposed workers (average 2.1 ppm) excreted considerably less MAA (average 5.4 mg/g Cr).

The values of Shih et al. (1999) and Chang et al. (2004) of 8 and 9 (end of Friday shift) and 4 (increase during week) mg/g Cr are consistent with those extrapolated from the Groeseneken et al. (1989) study of 8-12 and 3-5 mg/g Cr, respectively.

### **Mechanism studies**

2ME had no effect when incubated with human erythrocytes, whereas 0.5 mM of MAA increased their osmotic fragility. When human erythrocyte membranes (ghosts) were incubated with MAA or 2ME, membrane-bound acetylcholinesterase ( $IC_{50} = 5.5$  mM) and ATPase ( $IC_{50} = 1.4$  mM) were inhibited by MAA but not by 2ME (Mori et al., 1989).

Simultaneous administration of a number of other substances (formate, acetate, glycine, glucose, serine, sarcosine) involved in the formation of pyridine and purine – which in turn are needed for synthesis of DNA and RNA – reduces or completely eliminates the malformed sperm and disruption of spermatogenesis caused by 2ME in experimental animals.

Addition of 10  $\mu$ M MAA, but not 1  $\mu$ M, reduced the proliferative capacity of foetal mouse liver cells in vitro, observed as reduced incorporation of tritium-labelled thymidine. However, no effect on survival of the cells was observed (Holladay et al., 1994).

Studies with bone marrow cells from a leukaemia patient without bone marrow involvement showed 50% inhibition after 24-h treatment with 3 mM MALD or 3.9 mM MAA. Similar results were obtained with a human leukaemia cell line (HL60). Caspase-3 enzyme activity, an effector of apoptosis, was greatly enhanced by MALD, and inhibition of caspase-3 attenuated MALD and MAA-induced cell death (Takagi et al., 2002).

### **Toxic effects**

#### *Animal data*

2ME and 2MEA have moderate acute toxicity. The reported  $LD_{50}$  values for 2ME range from 0.9 to 3.4 g/kg body weight, depending on species and method of administration. The reported  $LC_{50}$  for inhalation is 4600 mg/m<sup>3</sup> (1480 ppm). Four hours of exposure to 1000 ppm resulted

in atrophied sperm in male rats, and 625 ppm produced damaged spermatids within 24 h. The reported LD<sub>50</sub> values for 2MEA range from 1.3 to 5.6 g/kg. The reported LD<sub>50</sub> for MAA with oral administration (in water) is 1 to 1.5 g/kg .

Short-term exposures via gavage, skin application, feed and inhalation have similar effects in several species, including reduced thymus, spleen and testes weights, lower counts of white and red blood cells and platelets, lower hematocrit, haemoglobin levels and bone marrow cellularity, higher numbers of immature granulocytes and disturbance of spermatogenesis. Spermatogenesis is disrupted at a particular phase, the late pachytene, and the effect shows up later in lower sperm counts or aspermia. Toxicity is about the same regardless of the method of exposure – gavage, drinking water, skin application or inhalation .

After tests with rabbits, 2ME and 2MEA were classified according to EEC criteria as non-irritating to skin, and 2ME as non-irritating to eyes .

#### *Human data*

No reports on skin irritation, eye irritation or sensitization were found in the literature.

Older studies report that repeated occupational exposure to products containing 2ME can cause headaches, weakness, dizziness, ataxia, toxic encephalopathy and dampened reflexes . Further case reports are summarized in Table 1.

In a cross-sectional study of 65 workers who produced and packaged 2ME, measured concentrations in workplace air were 4 to 20 ppm and personal monitors indicated 5.4 to 8.5 ppm (time-weighted averages). Trends (not significant) to lower leukocyte counts and lowered haemoglobin were seen in the 40 exposed workers when they were compared with the 25 unexposed workers. Closer study of a sub-group of 6 exposed and 9 unexposed workers showed tendencies to reduced leukocyte counts, lower haemoglobin, reduced testicle size, lower sperm counts, elevated levels of luteinizing hormone (LH) and lower levels of testosterone and follicle-stimulating hormone (FSH) in serum, none of which was statistically significant (Cook et al., 1982).

Of 73 painters at a shipyard, 10% had anaemia and 5% had granulocytopenia, compared with 0% in an unexposed control group. No other haematological differences between the groups were observed. Measured exposure levels were 0-5.6 ppm for 2ME (geometric mean 0.35 ppm, n=81 personal samples) and 0-21.5 for 2-ethoxyethanol (2EE, ethylene glycol ethyl ether, EGEE) (geometric mean 2.6). A review of patient journals revealed that the conditions had arisen during employment as painters. The authors listed about 60 substances that painters at a shipyard might be exposed to, and of these lead, benzene and glycol ethers were identified as potentially harmful to blood-forming organs. All blood-lead levels were below 40 µg/dl and most of them were below 20 µg/dl. Air monitoring and product reviews indicated negligible exposure to benzene, and the authors concluded that the observed haematological effects could not be explained by exposure to lead or benzene (Sparer et al., 1988, Welch and Cullen, 1988, Welch et al., 1988).

Haematological effects were investigated in 53 impregnation workers from two copper clad laminate factories mainly exposed to 2ME were compared with a control group of 121 lamination workers with little, indirect exposure to 2ME. The raw material used in the plants included epoxy and phenolic resins, dicyanamide, 2-methylimidazole, antimony, aluminum, titanium and silica oxides, pigments, acetone and 2ME. Acetone and 2ME were the only volatile substances. The solvent contained 30% acetone and 70% 2ME. The average

exposures to 2ME in the impregnation area were 4.0 ppm (n=55 personal samples) and 4.3 ppm (n=11), respectively, in the two factories. The exposure in the lamination area (n=9) ranged from non-detectable to 0.28 ppm. The corresponding values for MAA in urine were: 20.0 and 20.9 (range 0-66) mg/g Cr in the exposed groups and 1.6 (0-4.2) mg/g Cr in the controls. Haemoglobin, packed cell volume, and red blood cell count in the 47 exposed male workers were significantly lower than in the 93 male controls. Further, the frequency of anaemia in the exposed group (26.1%) was significantly higher than in the control group (3.2%). No differences were found between the 6 exposed female workers and the 27 female controls. Using a multiple regression model with adjustment for sex, body mass index, and duration of employment, red blood cell count was significantly negatively associated with air concentrations of 2ME. Haemoglobin, packed cell volume, and red blood cell count were significantly negatively associated with urinary concentrations of MAA (Shih et al. 1999). This study shows clear evidence of haematologic effects in males at an average exposure of about 4 ppm 2ME and an average MAA level in urine of about 20 mg/g Cr. However, individuals in control group were also exposed to 2ME and effects among these cannot be ruled out.

In a follow-up survey of haematological effects, 29 exposed and 90 non-exposed workers were recruited. Haematological parameters, full-week, full shift personal exposure to 2ME, and urinary MAA were repeatedly measured in three consecutive surveys within six months. The mean airborne exposure of 2ME in the three surveys dropped from 9.6 to 2.3 ppm after 2.5 months and to 0.34 ppm after 6 months (geometric means). For comparison, the average exposure in the control group was 0.08 (gm, n=9, range n.d-0.8 ppm). In the first exposure survey haemoglobin, packed cell volume, and red blood cell count were significantly lower in the male exposed workers than those in controls, whereas lymphocyte and platelet counts were increased. The haemoglobin content of the erythrocytes was increased in the exposed (significant increases in MCV, MCH, and MCHC) suggesting disturbed blood formation. The frequency of anaemia was also significantly higher among the exposed (42% versus 3% among controls). The haematological effects were significantly associated with the urinary MAA of exposed workers. The haematological effects had returned to normal in the first follow up survey and remained normal in the second follow-up. The authors concluded that 2ME caused the haematological effects and that the reduction in exposure through both inhalation and potential dermal contact accounted for the recovery (Shih et al., 2003). This study suggests that the haematological effects of 2ME are reversible. However, the exposure levels in the follow-up measurements should not be interpreted as NOAELs, since the apparent lack of effect may be due to over-compensation in blood formation when exposure levels are reduced, or that the control group was also exposed to 2ME. Further, the authors do not present statistical analyses of the haematological effects at the follow-up occasions.

### **Mutagenicity**

With the exception of the studies reviewed below, 2ME and its metabolite MAA have been negative in all genotoxicity studies, including Ames tests, in all tested *Salmonella* strains, both with and without addition of metabolizing systems (for review, see McGregor, 1996).

2ME caused mutations in the *gpt* gene in a cell line from Chinese hamsters, but no mutations in the *hprt* gene in another cell line. The metabolite MALD was weakly mutagenic in *Salmonella* (TA97a) and gave rise to an increased number of mutations, sister chromatid exchanges and chromosome aberrations in Chinese hamster cells in vitro, in the concentration interval 5 – 40 mM. Chromosome damage from MALD was also seen in human lymphocytes

after 1 h at 40 mM and after 24 h at 2.5 mM. No chromosome damage was observed in mice that had been given up to 1000 mg/kg MALD or up to 2500 mg/kg 2ME by gavage.

2ME and its metabolites were tested for genotoxicity and epigenetic effects in different test systems. Increased numbers of micronuclei and mitotic irregularities were seen in vitro at 65 mM 2ME, 0.12 mM MALD and 3.2 mM MAA. With MALD, an elevated frequency of mutations was seen at 1 – 10 mM, of sister chromatid exchanges and chromosome aberrations at 0.1 – 1 mM, and of morphological transformations at 0.1 – 0.3 mM. The authors regard the results as weakly positive for 2ME and MAA and clearly positive for MALD (Elias et al., 1996).

2MEA was tested with a number of Salmonella strains, both with and without metabolic activation, in two different laboratories. One judged it to be weakly mutagenic, and the other judged it to be possibly mutagenic (Zeiger et al., 1992).

## **Carcinogenicity**

There are no reports of animal studies on the carcinogenicity of 2ME or 2MEA. According to a review by McGregor (1996), aside from a few positive results in Ames tests (see section on Mutagenicity), there is no experimental evidence that either of the substances is carcinogenic.

A review of 198 cases of acute myelotic leukaemia (AML) was made in a French case-control study. Blind estimates of exposure to different types of glycol ethers and potential exposure levels were made by an expert panel. No relationship between AML and glycol ethers was seen (Hours et al. 1996).

## **Reproduction toxicity**

### *Animal data*

A large number of animal studies have provided a clear and unambiguous picture of the toxic effects on reproduction in both sexes. Males given low doses have reduced testes weights, histological changes in testes and low sperm counts; higher doses cause testicular atrophy and aspermia. The effects are transient. Females have lower fertility and higher numbers of dead and resorbed fetuses. The offspring has lower post-natal survival rate and higher frequencies of skeletal anomalies, malformed extremities and severe deformities. Effects on fetuses appear at doses with no visible maternal effects. At higher doses there is 100% foetal mortality. The degree of foetal damage is extremely sensitive to the time of exposure. These effects have been demonstrated for all administration methods and in several different species. The critical study appears to be that of Hanley et al. (1984). They exposed rats, rabbits and mice to 3, 10 or 50 ppm 2ME for 6 h/d during gestation. The NOEL was 10 ppm, although delayed ossification and increased resorption, judged to be unrelated to exposure, was seen in rabbits at this level. At 50 ppm (LOEL) reduced maternal weight gain, skeletal variations in offspring, and slight foetotoxicity was seen. In rabbits, increased resorption, skeletal and soft tissue variations, and malformations in 91 of 145 fetuses were seen. A few of the more recent studies are summarized below.

Rabbits were given 2ME in drinking water, 12.5 – 50 mg/kg/day, 5 days/week for 12 weeks: there were dose-dependent declines in several parameters of sperm quality. The effects were



significant at 37.5 and 50 mg/kg, and the most marked effect was reduced number of sperm per ejaculate. Histological examination revealed that spermatogenesis (number of round spermatids per Sertoli cell) was somewhat reduced at 25 and severely disrupted at 37.5 mg/kg. At the highest dose, 50 mg/kg, spermatogenesis ceased almost completely in 5 of 7 rabbits. No effects were seen on the libido or fertility of the rabbits that still had functional sperm production, and no other pathological or histopathological effects were observed. The authors concluded that spermatogenesis in rabbits is about 10 times more sensitive to 2ME than that in rats or mice (Berndtson et al., 1997, Foote et al., 1995).

2ME given to female rats in doses of 300 mg/kg/day completely eliminated the oestrus cycle: inhibition of ovulation, luteal body hypertrophy, permanently elevated progesterone levels and permanently low levels of estradiol, follicle-stimulating hormone (FSH), luteinizing hormone (LH) and prolactin. Addition of MAA to luteal cells in vitro resulted in elevated progesterone levels in the cultivation medium at all levels; 1 mM was the lowest tested concentration (Davis et al., 1997). MAA was also tested in an in vitro system with luteinized granulosa cells from humans. Incubation with 0 – 5 mM of MAA for 6 to 48 h yielded a duration- and concentration-dependent increase of progesterone. The effect was significant at 1 mM, but a tendency was also apparent at 0.1 and 0.5 mM. The authors associate these observations with the effects on menstrual cycle and ovarian function in humans (Almekinder et al., 1997).

Pregnant monkeys (*Macaca fascicularis*) were given 2ME by gavage in doses of 12, 24, or 36 mg/kg/day on days 20 to 45 of gestation (during organogenesis). Effects on the mothers (moderate to extreme loss of appetite, weight loss) were observed at all dose levels. Most of the animals in the two higher dose groups were therefore given nourishment and/or electrolytes by gavage. Caesarean sections were performed after 100 days: all 8 fetuses in the high-dose group were dead or resorbed, as were 3 of 11 in the middle group and 4 of 14 in the low-dose group. This can be compared with 0 of 6 in the untreated control group and 0 of 3 in a control group treated with ethanol (0.47 mmol/kg/day). The authors noted that the dead fetuses looked different from those seen in spontaneous, drug-induced or ethanol-induced foetal deaths, and drew the conclusion that the effect was not secondary to the maternal toxicity but a direct effect of the 2ME. One of the dead fetuses in the highest dose group had no digits on the forelimbs. Deformities of this type had not previously been seen in monkeys, but had been observed earlier in mice and rabbits given 2ME. Other deformities were also observed, but the possibility that these were secondary to the death of the foetus could not be ruled out. No deformities were seen in the living foetuses (Scott et al., 1989).

The addition of 10 µM MAA reduced mitosis in foetal liver cells in vitro (Holladay et al., 1994). According to a toxicokinetic model, this level is equivalent to 8 h of inhalation exposure to 1 ppm 2ME (Welsch et al., 1995).

#### *Human data*

In examination of sperm quality in workers at a shipyard, it was found that painters had oligospermia (10/79 compared to 0/40) and azoospermia (4/79 compared to 0/40) more often than controls, as well as a tendency to a lower number of sperm per ejaculate. Average exposures of the painters were 0.8 ppm 2ME and 2.6 ppm 2EE. Urine analyses of the 2EE metabolite ethoxyacetic acid indicated considerable skin exposure (Welch et al., 1988).

In a previously cited study of copper clad laminate factories, no significant differences in sperm parameters (volume, sperm count and morphology), except a lower pH in semen, was

seen between 14 highly exposed (4.0-4.2 ppm) impregnation workers and 13 less exposed (<0.28 ppm) laminate workers (Shih et al. 1999).

A woman whose job during two pregnancies was to rinse laboratory glassware in 2MEA gave birth to boys with genital defects (hypospadias, micropenis, bifid type of scrotum) in both cases. The authors could find no other factors in the work environment, the home environment or heredity that could explain them (Bolt and Golka, 1990).

There is a report on 44 patients in Matamoros, Mexico, who had a syndrome with characteristic facial distortions and mental retardation. All of them were born in 1971 – 1977 and were children of mothers who during their pregnancies had worked at a factory making condensers. There are no quantitative data on exposure, but during work the women had dipped their hands into a solution consisting mainly of 2ME and ethylene glycol. There was no ventilation, and workers used no protective gloves or face masks. Indications of acute poisoning, with fatigue, dizziness, nausea and vomiting, had occurred during work. A closer examination of 28 of the cases revealed that all of them also had musculoskeletal defects and that about half of them had eye and ear defects as well. There was no familial relationship between the cases and birth defects of this nature had not occurred previously in any of the affected families (Saavedra et al., 1997).

An elevated frequency of spontaneous abortions (compared with unexposed controls) was noted in women in the semiconductor industry, and was associated particularly with diffusion/dipping and photolithography. (For a brief description of the production of integrated circuits, see e.g. Britannica Online, 1999). Exposure to glycol ethers, xylene, toluene and hexamethyl disilazane was reported to occur during photolithographic work, and to arsine, phosphine and diborane during diffusion work. No exposure measurements were made (Pastides et al., 1988). This report served to initiate several further epidemiological studies in the semiconductor industry.

In a cohort study of 6088 women employed in 14 semiconductor factories, 904 pregnancies and 113 miscarriages were examined. After control for age, smoking habits, ethnic background, education, income, date of pregnancy and stress levels, there was a tendency for women in production work to have a higher proportion of miscarriages than other employees. A significantly higher frequency was seen among women who worked with masking. In this group, the highest risk of miscarriage was associated with etching (Beaumont et al., 1995). In a sub-study, the outcomes of 891 pregnancies were sorted according to exposure during the first trimester. Women working with photolithography, who were exposed to ethylene glycol ethers (2ME, 2EE and their acetates), fluorides and other substances, had a significantly higher risk of miscarriage (Swan et al., 1995).

In a prospective study made at the same companies, 403 women were followed for 6 months by analysis of chorionic gonadotropin in urine. After control for possibility of conceiving, use of contraceptives and age, there was significantly lower fertility in the women working in dipping and the same tendency in those exposed to glycol ethers. In addition, female production workers had a significantly higher risk of spontaneous abortions than those who held other types of jobs. All three pregnancies among the women who were exposed to ethylene glycol ethers terminated in miscarriages (Eskenazi et al., 1995). The same group of women also kept diaries on their menstruations. Prolonged menstrual cycles were seen in women who worked with dipping, and shortened cycles and a greater number of irregular menstruations were seen in the photolithography group (Gold et al., 1995).

In exposure assessments made in the workplaces at the same time, it is stated that 15 – 20% of

the factories used photo chemicals (negative photo resist), usually containing 3% 2ME. All personal monitors registered 2ME levels below 10 ppb, average exposure to 2-ethoxyethyl acetate (2EEA, ethylene glycol ethylether acetate, EGEEA) was 22 ppb, and exposure to 1-methoxypropyl acetate was 8 ppb (Hammond et al., 1996). Exposures to glycol ethers were strongly correlated to exposures to xylene and n-butyl acetate (Hines et al., 1996).

In a study of 454 pregnancies among 1368 women employed in the semiconductor industry, risk of spontaneous abortion tended to be higher for those who worked in chip production, and those with chemical exposure outside of chip production, than for unexposed subjects. The proportion of stillbirths also tended to be higher in the two exposed groups. The authors report that chip production involves exposure to glycol ethers and a number of other solvents, which they listed, but exposures were not measured (Pinney and Lemasters, 1996).

Another study in the semiconductor industry covered both female employees (561 pregnancies) and the wives of male employees (589 pregnancies). For the female employees, those with the highest likelihood of exposure to ethylene glycol ethers had significantly reduced fertility and elevated risk of spontaneous abortion. No increased risk of miscarriage was found among the wives of employed men, but there was a tendency to lower fertility. No personal monitoring measurements were taken, and only general information on exposures is given. A few measurements yielded glycol ether levels below 0.2 ppm in the highest exposure group. The glycol ethers named in the study are diethylene glycol dimethylether (DEGDME) and 2EEA; 2ME was not mentioned. Simultaneous exposure to glycol ethers and hexamethyl disilazane occurred. No increase in frequency of the studied effects was noted with exposure to n-butyl acetate, N-methyl-2-pyrrolidone or xylene, unless there was simultaneous exposure to glycol ether (Correa et al., 1996).

In all the referred epidemiological studies the authors claim there was exposure to no factors, other than glycol ethers, known to have toxic effects on reproduction. However, none of the studies made in the semiconductor industry contains detailed information on exposure levels. About 400 air samples were analyzed in a separate study, and average levels of 0.1 ppm 2ME and 0.01 ppm 2MEA were found (Paustenbach, 1988).

## **Immunotoxicity**

### *Animal data*

All clinical, morphological and histological indications of leukaemia disappeared in male rats that had been given subcutaneous injections of human leukaemia cells when they were given drinking water containing 2.5 mg/ml 2ME. Addition of 0.25 mg/ml, equivalent to a daily dose of 15 mg/kg, halved the leukaemia response. 2EE also retarded the leukaemia response, but was ten times less potent than 2ME. Seven other tested glycols and glycol ethers had no effect. In vitro tests with the same cell line showed a concentration-dependent reduction in number of cells in the dose interval 1 – 100  $\mu$ M 2ME. The metabolite MAA was about half as effective, which the authors regard as an indication that the mitosis-inhibiting effect of 2ME is not due to a cytotoxic mechanism alone (Dieter et al., 1990).

Mice given 2ME by gavage in doses of 500 or 100 mg/kg/day for 5 to 10 days developed atrophy and decline in mature thymocytes in the thymal cortex, but the medulla was unaffected (Kayama et al., 1991).

Female rats were exposed to 2ME in drinking water, 2000 or 6000 mg/l (equivalent to 161 or

486 mg/kg/day), for 21 days: the treatment resulted in a dose-dependent reduction of thymus weight, increased activity of killer cells, reduced antibody production and a lower number of cells in the spleen. At 6000 mg/l there was also reduced production of gamma interferon. Male rats exposed to 1600 or 4800 ppm in drinking water (200 or 531 mg/kg/day) showed all these effects as well as reduced testes weights at both dose levels. Thymus atrophy and reduced interleukin-2 production were also seen at the higher dose (Exon et al., 1991).

Single oral doses of 125 and 500 mg/kg caused a 3 and 8 times higher apoptotic index in the thymus, compared with unexposed rats. There was a parallel increase in the capacity of the liver to metabolize MALD to MAA. Pre-treatment with phenobarbital suppressed this effect almost completely (Balasubramanian et al., 1995).

Immune response was studied in rats and mice that had been given 2ME, 2MEA, MALD or MAA in oral doses of 50 to 400 mg/kg/day for 10 days. In the rats, the four substances yielded similar immunosuppression, expressed as reduced thymus and spleen weights and reduced antibody plaque-forming cell (PFC) response. The effects were significant at the lowest dose level, and equimolar doses of the four substances produced equivalent immunosuppression. Pre-treatment with 4-methylpyrazole caused these effects to disappear, which indicates that metabolic activation is required. This immunosuppression was observed in all of the rat strains but in none of the mouse strains. Nor did MAA in subcutaneous doses of up to 1920 mg/kg/day produce immunosuppression in the mice, which indicates that the difference between the species can not be explained by differences in bioavailability or metabolic rate .

Atrophy, dose-dependent reduction in cellularity, and changes in thymocyte patterns indicating disturbances in thymocyte maturation were observed in thymus glands from the young of mice given 2ME in doses of 100 – 200 mg/kg/day on days 10 to 17 of gestation (Holladay et al., 1994).

There are a number of other animal studies that support these findings of immunotoxic effects of 2ME.

#### *Human data*

Effects on several kinds of leukocytes were observed in 9 floor layers when they were compared with an unexposed, matched control group. The changes comprised reduced numbers of eosinophils and segmented neutrophils and increased numbers of rod neutrophils and lymphocytes. Among the lymphocytes there were lower numbers of T cells and helper cells, but higher numbers of NK and B cells. According to the authors, this lymphocyte pattern resembles the one seen in immune-deficiency diseases. Tendencies to lower haemoglobin values and lower numbers of erythrocytes were also observed. The floor layers were exposed to a number of solvents, including 2ME (mean 6.1, maximum 150 mg/m<sup>3</sup>), 2EE, 2-butoxyethanol, butanol, isobutanol, toluene, xylene, methyl ethyl ketone and methyl isobutyl ketone. Solvent levels in blood indicated that 2ME was the predominant exposure (Denkhaus et al., 1986).

Sweeney and colleagues (2001) applied a previously developed PBPK models (Gargas et al. (2000) and Monte Carlo simulations to derive health-based OELs for 2ME and other ethylene glycol ethers. The model was used to calculate estimated human-equivalent NOAELs, based upon internal concentrations in rats exposed to NOAEL doses for developmental toxicity. An estimated NOAEL value of 12 ppm for 2ME was derived using average values for the PBPK model parameters. The uncertainty in this point estimate was estimated from the distribution

of internal dose estimates obtained by varying key model parameters over expected ranges and probability distributions. Using the 95th percentile value, the simulations suggested an uncertainty factors to account for variability in toxicokinetics among humans of 1.7 for 2ME, which is less than the default value of 3.3. (only remaining paragraph from the now deleted Dose-response chapter. Not very useful information - delete it?)

## **RECOMMENDATION**

Judging from both animal data and experience of occupational exposures, the critical effects of 2ME are its toxic effects on reproduction and blood formation.

Increased foetal death was observed in monkeys given 2ME in oral doses of 12 mg/kg/day during gestation (Scott et al., 1989). At 25 mg/kg/day there were effects on testes and sperm in rabbits (Berndtson et al., 1997, 16), and in rats prolonged gestation, smaller litters and deformed pups. Doses of 50 mg/kg/day affect the thymus, suppress the immune response, are toxic and teratogenic to foetuses of rodents and completely eliminate spermatogenesis in rabbits. At doses around 100 mg/kg/day these effects are considerably stronger, and bone marrow depression, disturbances in haematopoiesis and reduced fertility are also seen. Inhalation exposure to 50 ppm has foetotoxic effects on rodents, producing skeletal aberrations and deformities. It is noteworthy that most of these effects are seen in all species and with all methods of administration, although the required exposure level varies. These variations can probably be partly explained by differences in study design. It is therefore difficult to give a single critical effect. Immunological effects of inhalation exposure have not been studied with modern methods. However, in several animal species, reprotoxic effects appear at the lowest doses. Using the results of Hanley et al. (1984) a NOAEL of 10 ppm for embryotoxic, foetotoxic, and teratogenic effects can be derived for rats, rabbits and mice.

Adverse effects associated with occupational exposure to 2ME are listed in Table 1.

Welch and colleagues (Sparer et al., 1988, Welch and Cullen, 1988, Welch et al., 1988) reported increased prevalence of anaemia and granulocytopenia in shipyard painters exposed to 0.35 ppm 2ME (geometric mean). These workers were also exposed to the related glycol ether 2-ethoxyethanol (geometric mean 2.6 ppm), and lead (most blood lead levels below 20 µg/dl) and benzene (exposure judged to be negligible) making the data difficult to use as a basis for an OEL. Similarly, a more recent study reported anemia in 26% of workers engaged in copper clad lamination and exposed exclusively to 2ME at average levels of about 4 ppm 2ME in air and 20 mg MAA/g Cr MAA urine MAA of (Shih 1999).

Several studies report elevated incidences of miscarriage, menstrual irregularities and low fertility among women in the semiconductor industry. To the extent that exposure to 2ME occurs, the air levels in this context would be below 1 ppm. However, as the contribution of 2ME in relation to other agents is not clear, these studies cannot be used to derive an OEL.

In view of the haematologic effects seen in workers at 4 ppm (Shih et al. 1999) which were no longer observed at an exposure level of 2.3 ppm (Shih et al. 2003), SCOEL recommends a health-based OEL of 1 ppm. This value also protects from reproductive effects in animals for which a NOEL of 10 ppm was derived in several species. No irritation or other immediate effects occur near this level, hence no STEL value is deemed necessary.

2ME (and 2MEA) is efficiently biotransformed to the active metabolite MAA. Biological monitoring of MAA in urine is preferable to monitoring of 2ME in ambient air since the former (1) captures dermal as well as inhalation exposure to 2ME and (2) more closely reflects the body burden of active metabolite. Due to the long half time (77 h), urinary MAA will accumulate over a few work weeks.

Extrapolation of a 4-h human exposure study at rest Groeseneken et al. (1989) suggests that in a worker exposed at rest for at least two weeks at 1 ppm 2ME, and with no additional dermal exposure, the urinary excretion of MAA will reach 8-12 mg/g cr at end of Friday shift in the following weeks. This is also supported by data from field studies.

Accordingly, the BLV is set to 8 mg MAA/g cr so as to match the OEL of 1 ppm. Urine should be sampled at the end of the last shift of the work week after at least two weeks at work.

2MEA is rapidly transformed to 2ME in the body, and in animal experiments the two substances are equally toxic. The health hazards of 2MEA should therefore be regarded as equivalent to those of 2ME. Accordingly, the OEL for 2MEA is also set to 1 ppm and the BLV to 8 mg MAA/g Cr.

2ME and its acetate ester 2MEA are efficiently absorbed via both inhalation and skin exposure. Skin penetration can account for a large portion of the total uptake if the skin is exposed to liquids or even vapours containing 2ME or 2MEA. A skin notation is thus clearly warranted.

No measurement difficulties are foreseen at the recommended OEL and BLV.

(table moved here from the now deleted Dose-response chapter)

**Table 1.** Effects on human health associated with occupational exposure to 2ME.

Exposure level, ppm	Exposure situation	Number of persons	Observed effects	References
am <sup>1</sup> 0.8, median 0.44, range 0-5.6	Shipyards painters, also exposed to 2EE: am 0.42, median 1.2, range 0-21.5 ppm	73 men	10% had anaemia and 5% had granulocytopenia (0% in controls), low sperm counts.	Sparer et al., 1988, Welch and Cullen, 1988, Welch et al., 1988
am 2, peak 48	Floor layers; also exposed to 2EE and other solvents	9 men	Higher numbers of rod neutrophils, lymphocytes, NK and B cells. Lower numbers of eosinophils, segmented neutrophils, T cells and helper cells. Tendencies to lowered Hb and erythrocyte counts.	Denkhaus et al., 1986
am 4.0-4.2	Copper clad lamination - impregnation	47 men, 6 women	Reduced Hb, red blood cell count and packed cell volume. 26.1% anemic workers vs 3.2% in control group.	Shih et al., 1999
Control group: n.d. - 0.28		Control group: 93 men, 28 women	No significant haematological effects in women.	
5 - 9	Production and packaging of 2ME	65 men	Tendencies to lowered white blood cell count, Hb, testicle size, sperm count and serum testosterone and FSH. Tendency to elevated leuteinizing hormone in serum (studied in different subgroups).	Cook et al., 1982
about 8	Manual cleaning, skin exposure	2 men	Bone-marrow depression, pancytopenia.	See Johanson, 2000
gm <sup>2</sup> 9.6	Copper clad lamination - coating	24 men, 5 women	Lowered Hb, packed cell volume, and red and blood cell count. Increased white blood cell, lymphocyte and platelet counts 42% anaemic workers vs 3% in control group.	Shih et al., 2003
Follow-up 3 mo later: gm 2.3				
6 mo later: gm 0.34				
Control group: n.d. - 0.28		Control group: 67 men, 23 women	No haematological effects 3 and 6 mo later.	
18 - 58	Production and cleaning of microfilm	1 man	Apathy, fatigue, increased need for sleep. Lowered Hb, hematocrit, red and white blood cell and platelet counts.	See Johanson, 2000
60 - 4000 (reconstruction)	Print shop cleaning; large surfaces, skin exposure	6 men	Symptoms of poisoning, CNS effects. Hypocellular bone marrow (examined in only one of the men)	See Johanson, 2000

<sup>1</sup> am = arithmetic mean

<sup>2</sup> gm = geometric mean

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## Appendix

Toxicokinetic calculation of MAA levels in urine. Departure points:

- Use one-compartment model in Berkeley Madonna software, assume time-invariant linear kinetics (no metabolic saturation, no enzyme induction, etc).
- Calculate MAA excretion in urine according to following conditions:
  - o Use individual values of pulmonary ventilation (7.0-9.8 L/min), pulmonary EGME retention (73-79%), and urine recovery (78.6-91.5%) and half time (66.1-89.7 h) of MAA as reported by Groeseneken et al. (1989) for controlled 4-h exposure (Groeseneken et al., 1989).
  - o Normalize actual exposure of each subject (15.6-16.3 mg/m<sup>3</sup>) is to 3.11 mg/m<sup>3</sup> (1 ppm).
  - o Assume mg MAA/g Cr is equivalent to µg MAA/min (i.e. urinary creatinine excretion is 1 mg/min)
- For each subject, simulate MAA in urine (mg/g Cr) for 4 weeks of exposure (5 d/wk x 8 h/d) at 1 ppm 2ME.

MAA in urine, mg/g Cr	1 <sup>st</sup> week of exposure	4 <sup>th</sup> week of exposure
Monday, before shift	0	5-7
Monday, after shift	1.7-2.7	6-9
Friday, after shift	6-9	8-12
Increase during work week	6-9	3-5

